

Supporting Information to 'The chemical synthesis of selenium-modified oligoribonucleotides and their enzymatic ligation leading to an U6 snRNA stem-loop segment'

*Claudia Höbartner and Ronald Micura**

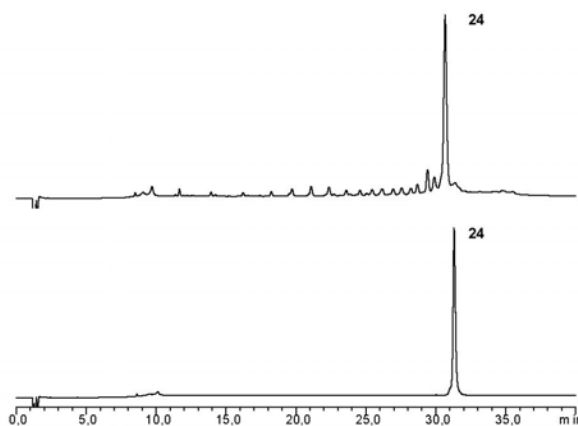
Oligoribonucleotide deprotection.

5'-AAGC_{Se}CACACAAACC(dA)(dG)(dA)CGGCC, 24

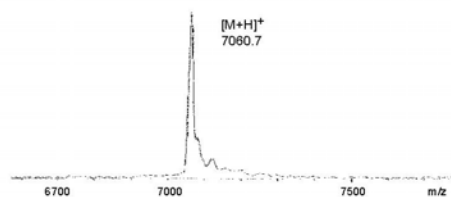
deprotection:

- 1) MeNH₂ 33% in EtOH / MeNH₂ 41% in H₂O, + 50 mM dithiothreitol, rt, 6h
- 2) 1M TBAF in THF, rt, 12h

purified oligoribonucleotide 24



MALDI TOF mass spectrum of 24

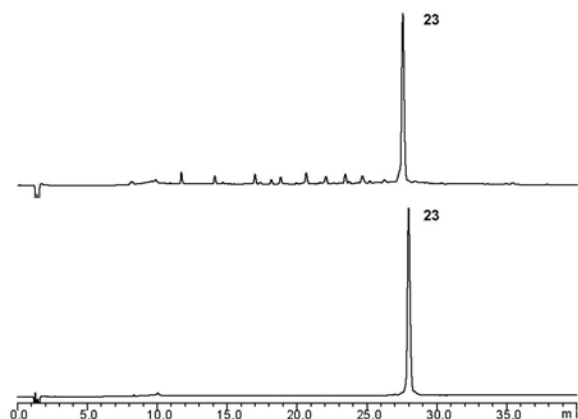


5'-Anthracene-(HEG)-GGAGCU_{Se}CGCC_{Se}C, 23

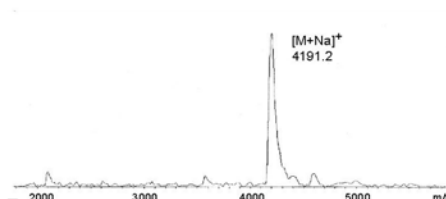
deprotection:

- 1) MeNH₂ 33% in EtOH / MeNH₂ 41% in H₂O, + 150 mM dithiothreitol, rt, 5h
- 2) 1M TBAF in THF, rt, 12h

purified oligoribonucleotide 23

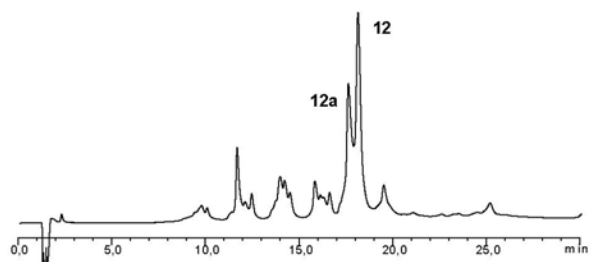


MALDI TOF mass spectrum of 23

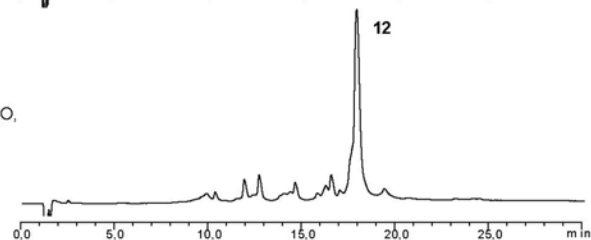


5'-CGCGU₅eGG, 12

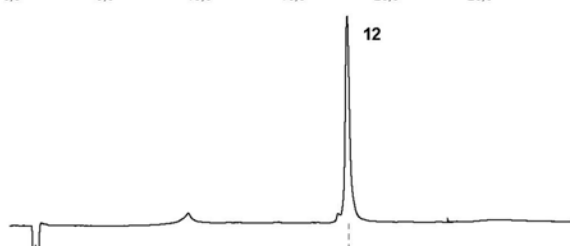
standard deprotection:
1) MeNH₂ 33% in EtOH, rt, 6h
2) 1M TBAF in THF, rt, 12h



optimized deprotection:
1) MeNH₂ 33% in EtOH / MeNH₂ 41% in H₂O,
+ 10 mM dithiothreitol, rt, 6h
2) 1M TBAF in THF, rt, 12h



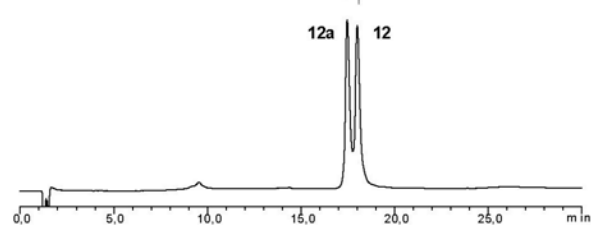
purified oligoribonucleotide 12



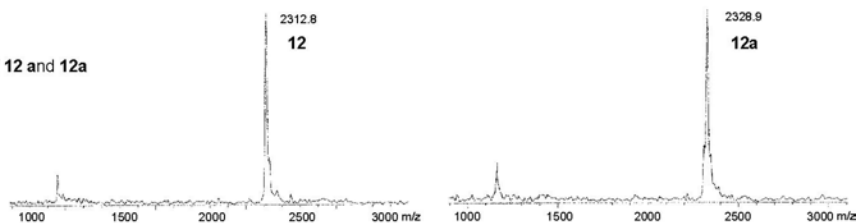
purified oligoribonucleotide 12a
(oxidation product)



coinjection of 12 and 12a



MALDI TOF mass spectra of 12 and 12a

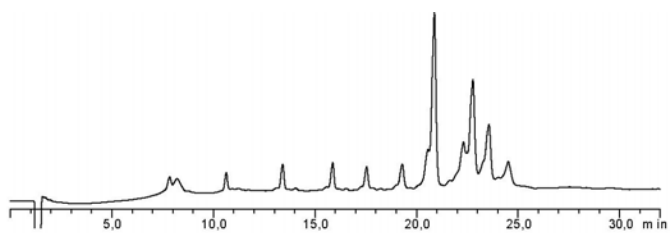


(see also Figure 1)

5'-G C₅₆GG CGG CGG C, 19

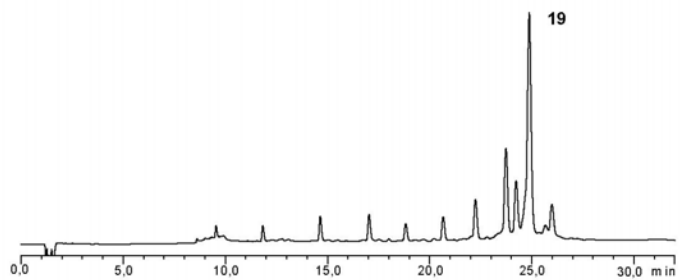
deprotection:

- 1) NH₃ in MeOH, 42°C, 20h
- 2) 1M TBAF in THF, rt, 12h

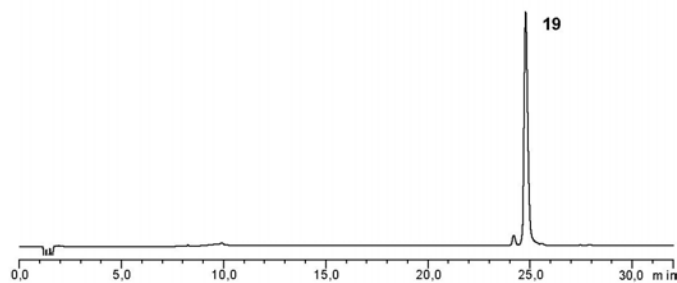


deprotection:

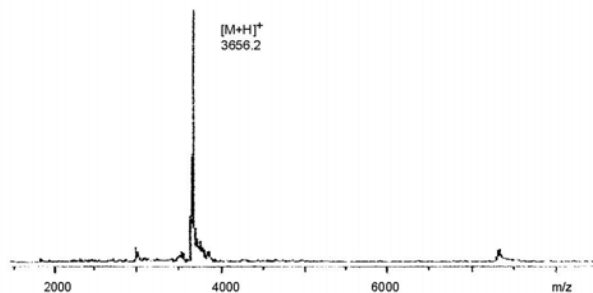
- 1) MeNH₂ 33% in EtOH / MeNH₂ 41% in H₂O, + 100 mM dithiothreitol, rt, 8h
- 2) 1M TBAF in THF, rt, 12h



purified oligoribonucleotide 19

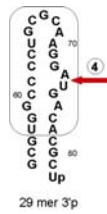


MALDI TOF mass spectrum of 19

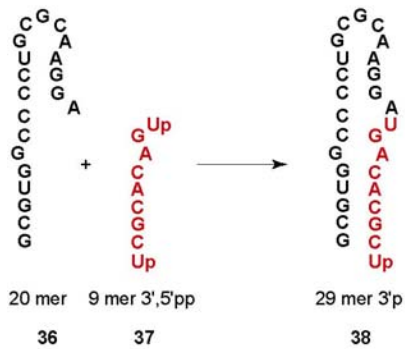


Enzymatic ligations using T4 RNA ligase.

Ligation site 4



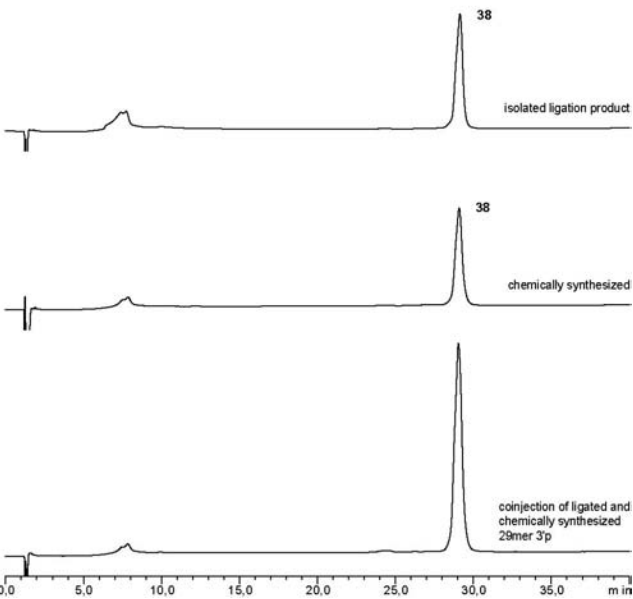
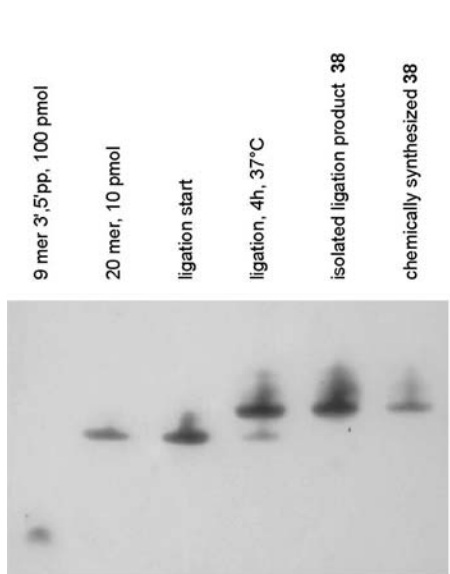
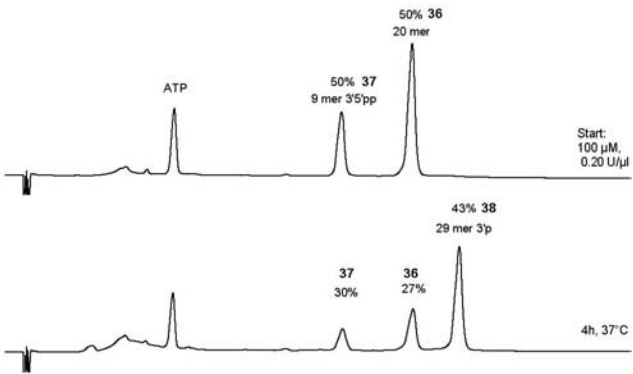
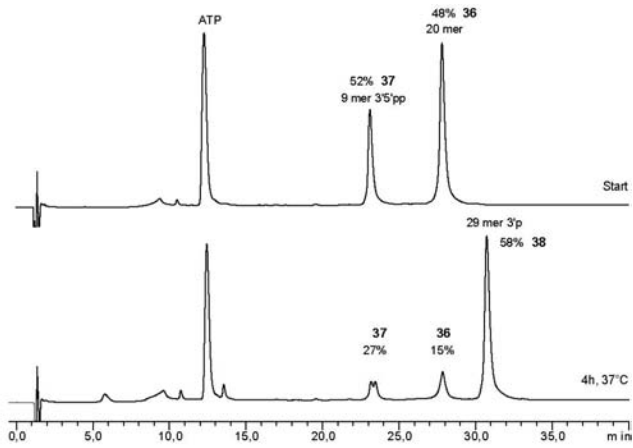
unmodified fragment oligoribonucleotides



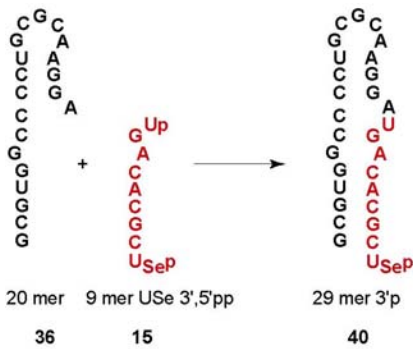
ligation conditions:
2 nmol per oligonucleotide, 40 μM in 1x ligation buffer,
10 U T4 RNA ligase, incubation at 37°C, 4h

preparative ligation conditions:
5-10 nmol per oligonucleotide, 92-106 μM in
1x ligation buffer, 10 U T4 RNA ligase,
incubation at 37°C, 4h;

calculated yield 43-56 %,
isolated yield: 37 %
(7.5 nmol starting from 20 nmol each)



3'-U_{Se}-modified donor oligoribonucleotide



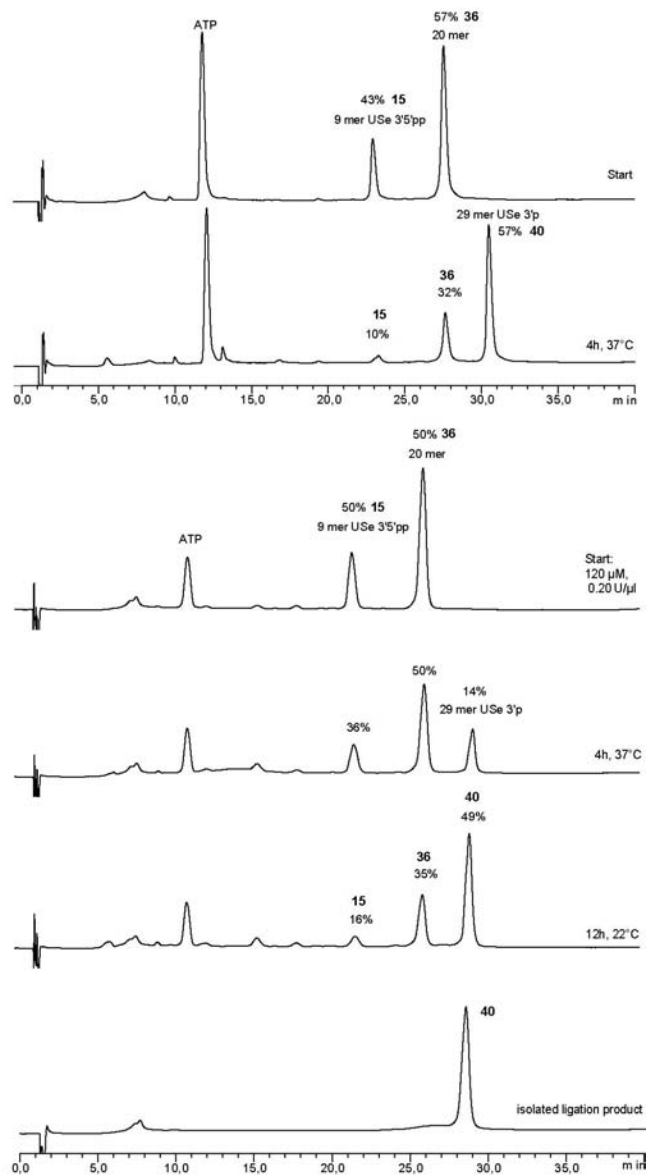
ligation conditions:
 2 nmol per oligonucleotide, 40 μM in 1x ligation buffer,
 10 U T4 RNA ligase, incubation at 37°C, 4h

preparative ligation conditions:
 10 nmol per oligonucleotide, 120 μM in
 1x ligation buffer

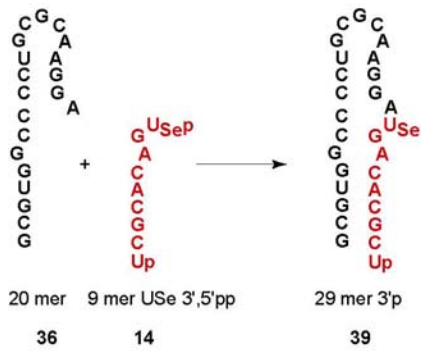
0.2 U/μl T4 RNA ligase,
 incubation at 37°C, 4h;
 calculated 14% ligation

finally 0.3 U/μl T4 RNA ligase,
 additional incubation at 22°C, 12h;
 calculated 49% ligation

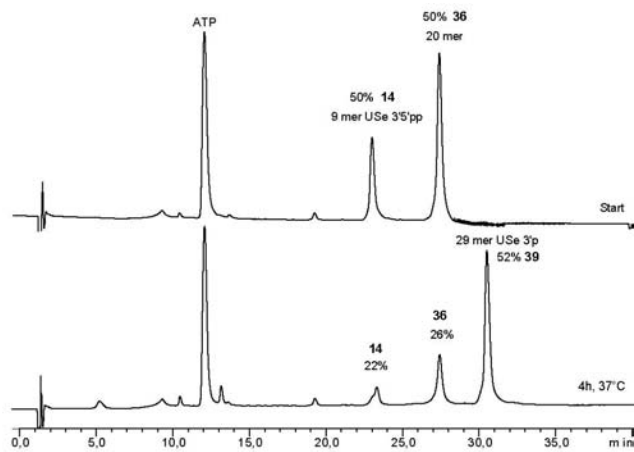
calculated yield 49-56%,
 isolated yield: 44%
 (8.8 nmol starting from 20 nmol each)



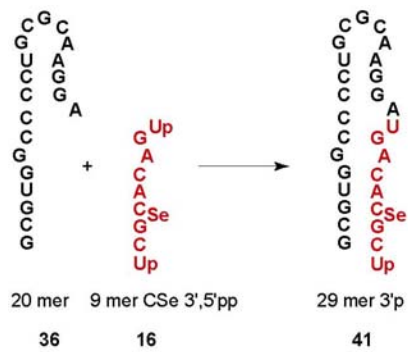
5'-U_{Se}-modified donor oligoribonucleotide



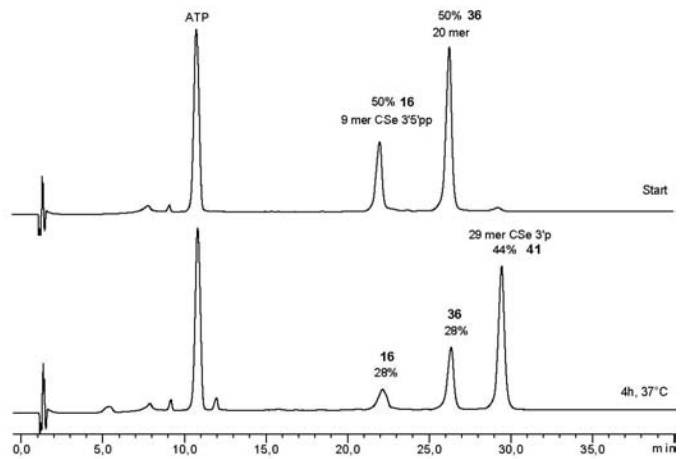
ligation conditions:
 2 nmol per oligonucleotide, 40 μM in 1x ligation buffer,
 10 U T4 RNA ligase, incubation at 37°C, 4h



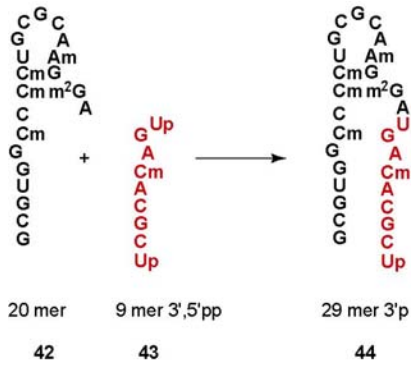
C_{Se}-modified donor oligoribonucleotide



ligation conditions:
 2 nmol per oligonucleotide, 40 μM in 1x ligation buffer,
 10 U T4 RNA ligase, incubation at 37°C, 4h



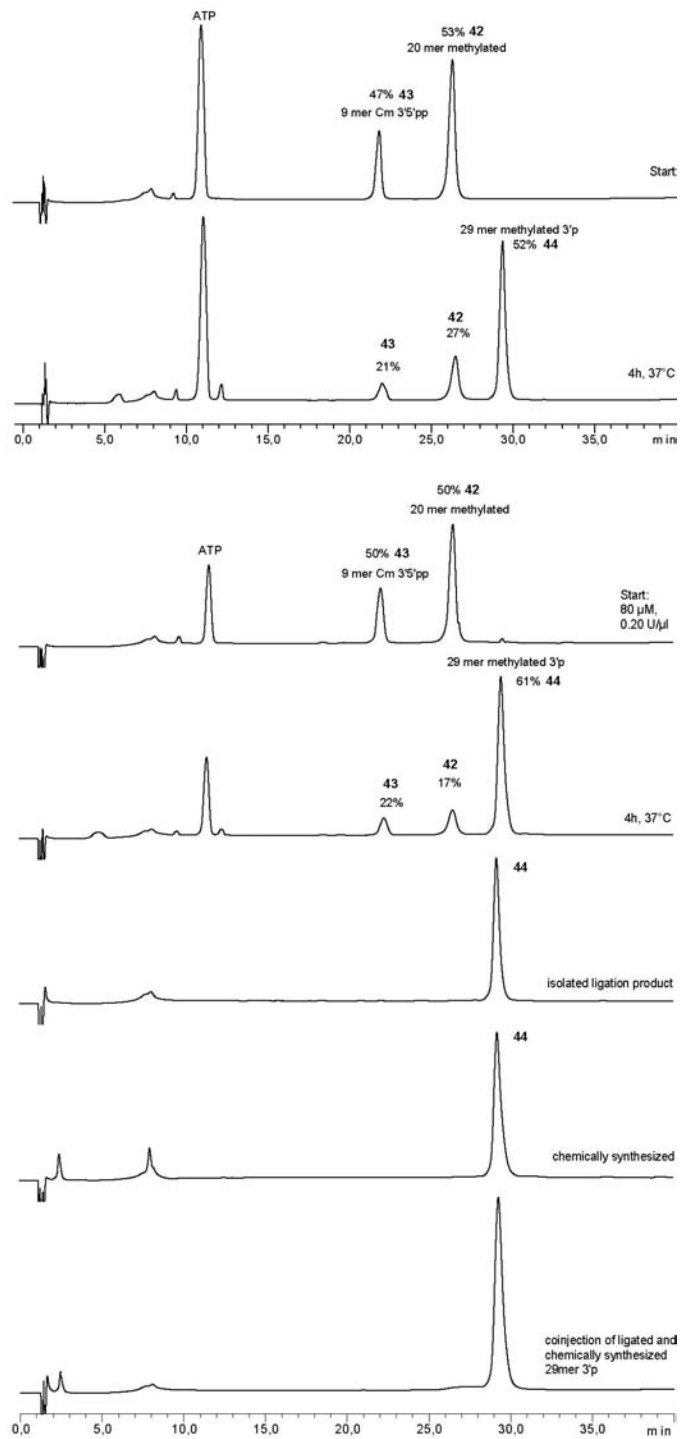
methylated fragment oligoribonucleotides



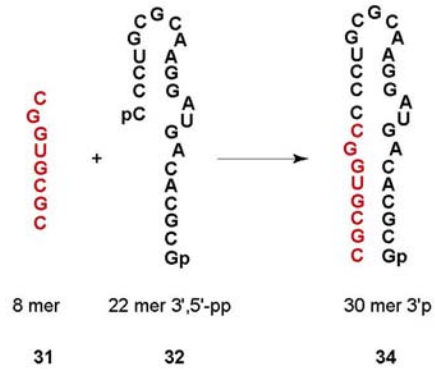
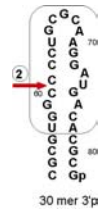
ligation conditions:
 2 nmol per oligonucleotide, 40 μM in 1x ligation buffer,
 10 U T4 RNA ligase, incubation at 37°C, 4h

preparative ligation conditions:
 5-8 nmol per oligonucleotide, 80 μM in
 1x ligation buffer, 10 U T4 RNA ligase,
 incubation at 37°C, 4h;

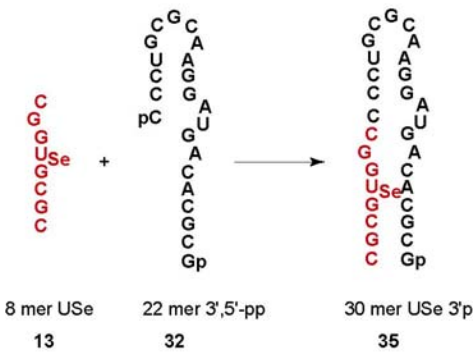
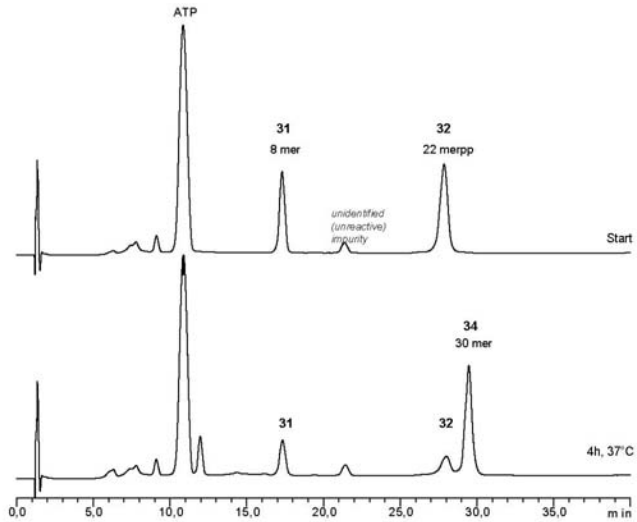
calculated yield 39-61%,
 isolated yield: 35%
 (3.5 nmol starting from 10 nmol each)



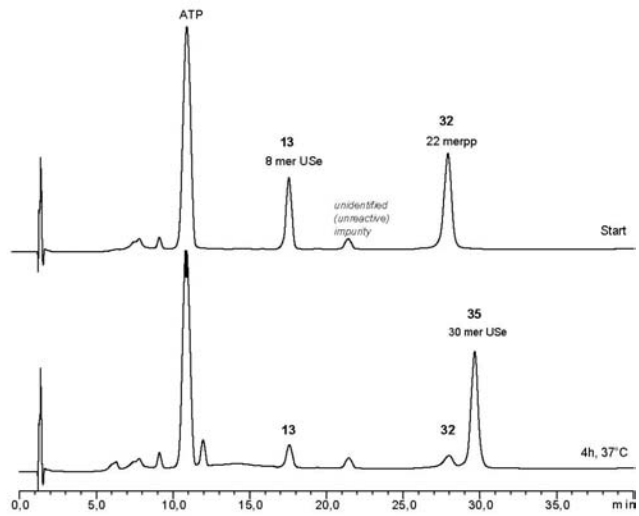
Ligation site 2



ligation conditions:
 1 nmol per oligonucleotide, 20 μ M in 1x ligation buffer,
 10 U T4 RNA ligase, incubation at 37°C, 4h



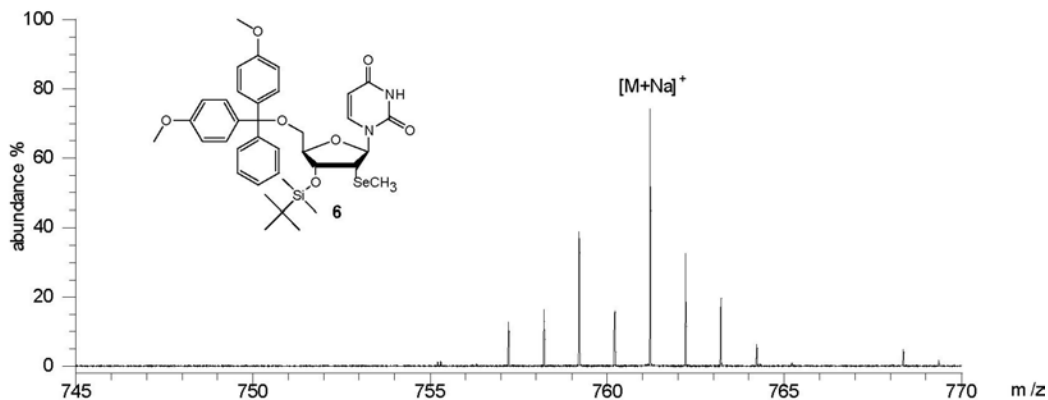
ligation conditions:
 1 nmol per oligonucleotide, 20 μ M in 1x ligation buffer,
 10 U T4 RNA ligase, incubation at 37°C, 4h



FT-ICR ESI MS spectra of compounds **6**, **8** – **11**.

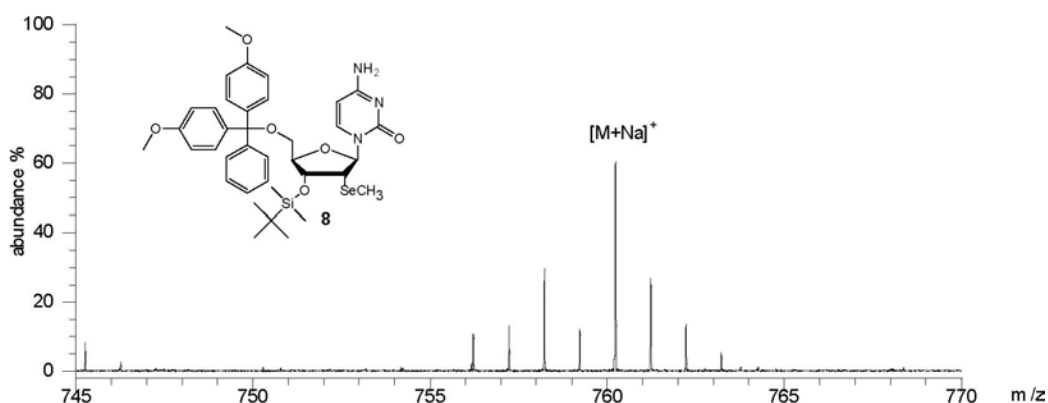
3'-O-Tert.-butyldimethylsilyl-5'-O-(4,4'-dimethoxytrityl)- 2'-O-deoxy-2'-Se-methyl-uridine (6)

FT ICR ESI-MS m/z calculated for $C_{37}H_{46}N_2O_7SeSi$ $[M+Na]^+$ 761.21421, found 761.21496.

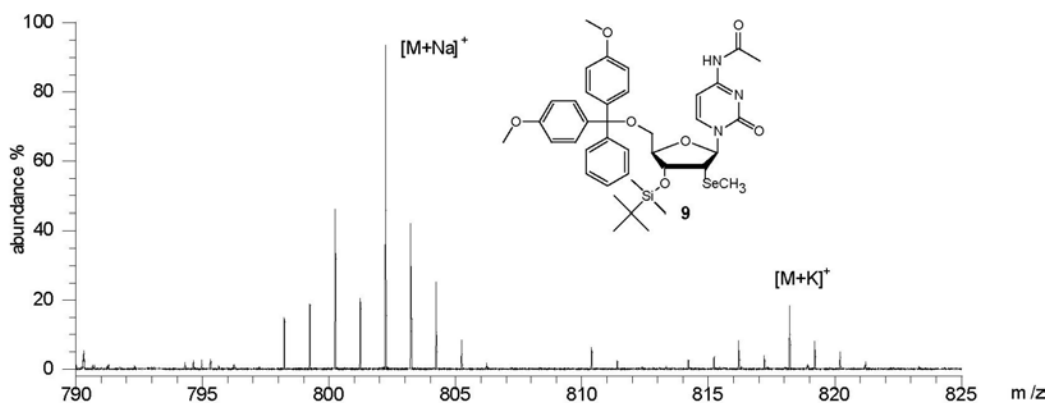


3'-O-Tert.-butyldimethylsilyl-5'-O-(4,4'-dimethoxytrityl)- 2'-O-deoxy-2'-Se-methyl-cytidine (8)

FT ICR ESI-MS m/z calculated for $C_{37}H_{47}N_3O_6SeSi$ $[M+Na]^+$ 760.23018, found 760.22880.

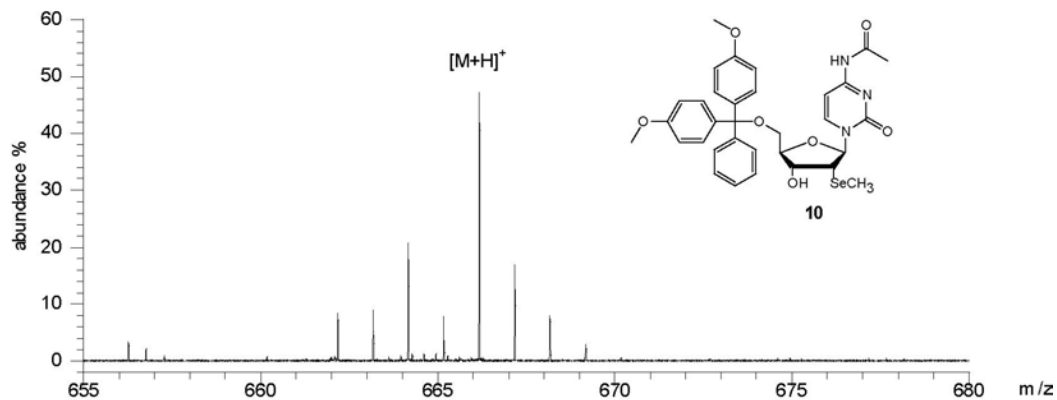


N⁴-Acetyl-3'-O-Tert.-butyldimethylsilyl-5'-O-(4,4'-dimethoxytrityl)- 2'-O-deoxy-2'-Se-methyl-cytidine (9) FT ICR ESI-MS m/z calculated for $C_{39}H_{49}N_3O_7SeSi$ $[M+Na]^+$ 802.24080, found 802.24121.



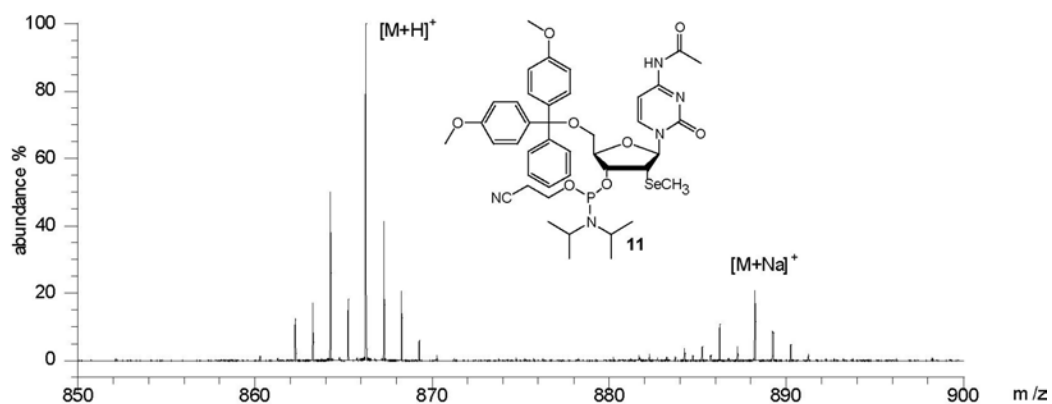
***N*⁴-Acetyl-5'-*O*-(4,4'-dimethoxytrityl)-2'-*O*-deoxy-2'-*Se*-methyl-cytidine (10)**

FT ICR ESI-MS *m/z* calculated for C₃₃H₃₅N₃O₇SeSi [M+ H]⁺ 666.17217, found 666.17208.



***N*⁴-Acetyl-5'-*O*-(4,4'-dimethoxytrityl)-2'-*O*-deoxy-2'-*Se*-methyl-cytidine 3'-*O*-(2-Cyanoethyl)-diisopropylphosphoramidite (11)**

FT ICR ESI-MS *m/z* calculated for C₄₂H₅₂N₅O₈PSe [M+ H]⁺ 866.28022, found 866.27866.



Representative trityl assays for strand assembly

Synthesis protocol for sequence 5'-CGCGU_{se}GGC, 13

Gene Assembler Plus

Date : 11/03/2003
Sequence : CH0344-1
Synthesis : CH0344-1
Sequence Length : 8
Column : 1
Final Detritylation : Yes
Coupling Efficiency Threshold : 75 %

Pos	Base	Retention mins	Duration mins	Peak ht %FS	Acc Area %min	Last eff %	Ave eff %
8	C	0.44	1.18	1645	215.89	-	-
7	G	0.36	0.98	1784	212.86	-	-
6	G	0.37	0.95	1778	211.05	99.1	99.1
5	U _{se}	0.40	1.21	1781	242.21	-	-
4	G	0.37	0.94	1767	209.10	99.5	99.3
3	C	0.43	1.27	1716	251.81	-	99.3
2	G	0.37	1.00	1771	205.21	99.1	99.3
1	C	0.43	1.19	1712	240.58	97.7	98.9

Total synthesis yield from start = 92.4 %

Synthesis protocol for sequence 5'-AAGC_{se}CACACAAACC(dA)(dG)(dA)C_{se}GGCC, 25

Gene Assembler Plus

Date : 14/07/2003
 Sequence : CH0380-4
 Synthesis : CH0380-4
 Sequence Length : 22
 Column : 1
 Final Detritylation : Yes
 Coupling Efficiency Threshold : 75 %

Pos	Base	Retention mins	Duration mins	Peak ht %FS	Acc Area %min	Last eff %	Ave eff %
22	C	0.43	1.42	1781	279.10	-	-
21	C	0.44	1.35	1761	312.34	-	-
20	G	0.37	0.89	1767	218.05	-	-
19	G	0.38	0.98	1775	231.08	106.0	100.0
18	C _{se}	0.43	1.20	1764	267.29	-	100.0
17	dA	0.42	1.26	1767	250.28	-	-
16	dG	0.42	1.02	1758	232.96	-	-
15	dA	0.41	1.18	1764	239.14	-	-
14	C	0.45	1.24	1708	259.70	97.4	100.0
13	C	0.47	1.30	1650	263.98	101.6	100.0
12	A	0.45	1.17	1690	239.73	-	100.0
11	A	0.46	1.20	1660	240.83	100.5	100.0
10	A	0.44	1.14	1691	229.59	95.3	100.0
9	C	0.46	1.29	1611	239.61	97.6	99.7
8	A	0.44	1.12	1660	221.05	98.1	99.5
7	C	0.45	1.26	1586	229.10	97.8	99.3
6	A	0.44	1.18	1634	215.88	98.8	99.2
5	C	0.46	1.34	1517	228.10	99.8	99.3
4	C _{se}	0.45	1.27	1580	215.01	98.5	99.2
3	G	0.40	0.98	1589	176.14	98.3	99.1
2	A	0.44	1.23	1488	193.60	97.3	99.0
1	A	0.45	1.07	1462	189.16	97.7	98.9

Total synthesis yield from start = 79.4 %